Automatic and Rapid Molecular Detection of *E. coli* and Enterococci in Raw Recreational Water Samples Using the Fully Automated Rheonix CARD® Technology Platform

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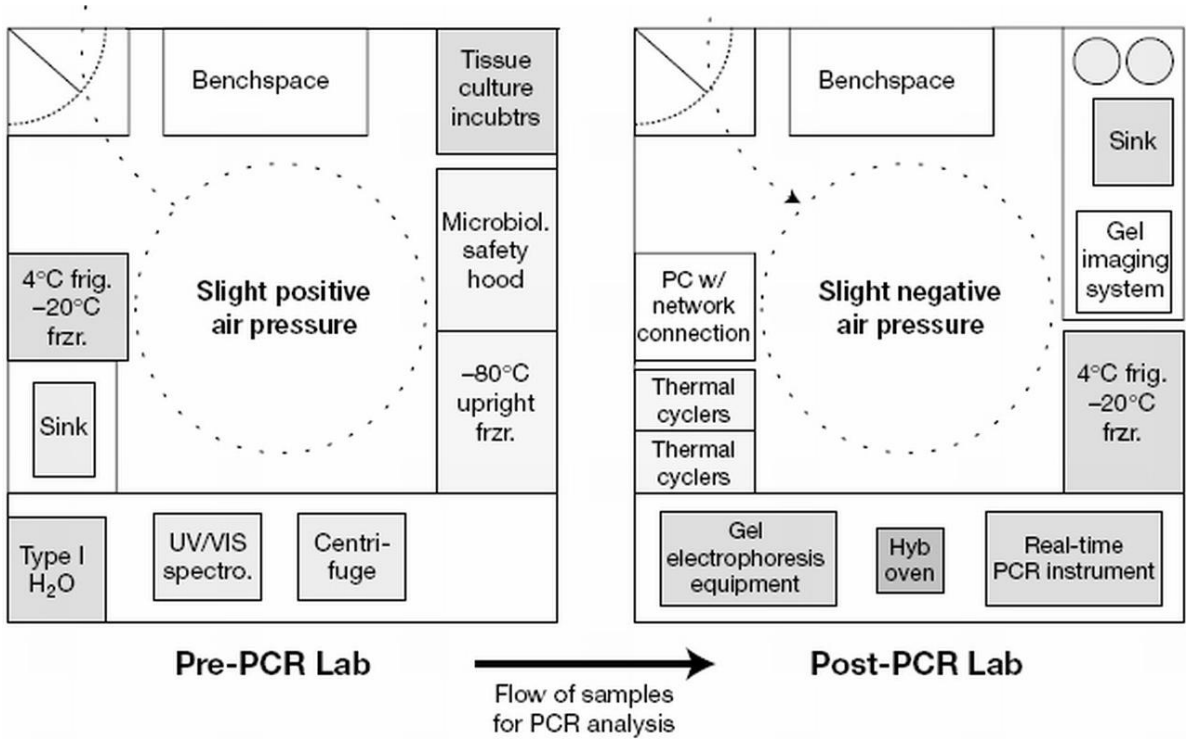
“Bench top” Molecular Diagnostics

- Sample acquisition
- Sample preparation
  - Cell concentration
  - Cell lysis
  - DNA/RNA isolation
- Gene amplification
  - PCR
  - Others
- Amplicon Detection
  - End point
  - Real time



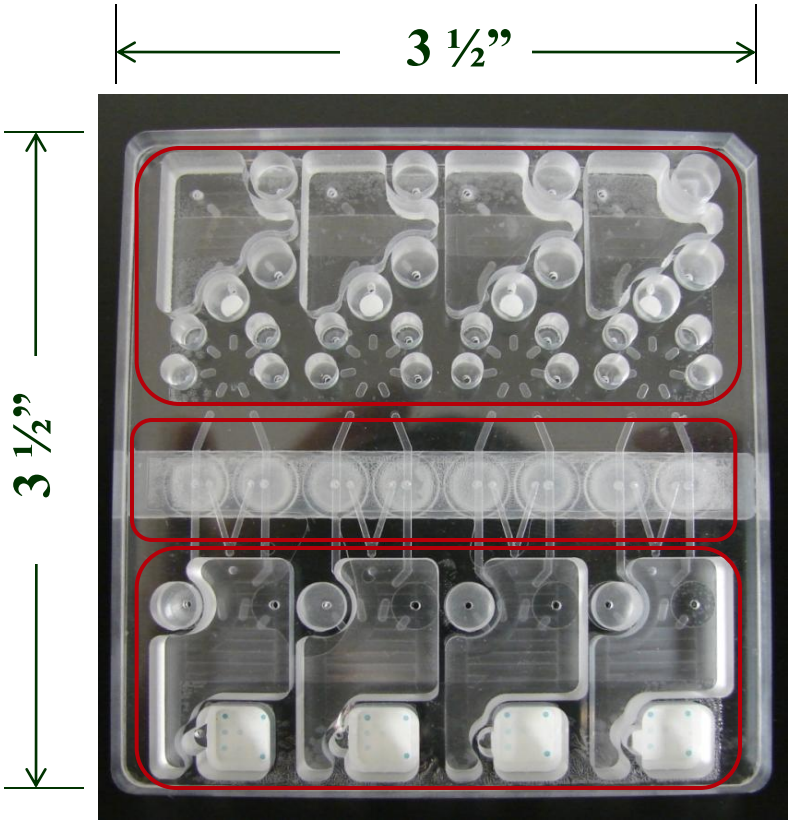
Rheonix has created a powerful microfluidic platform for the evolving molecular diagnostics industry. This system incorporates low cost disposable CARD® technology that can analyze single or multiple raw clinical samples. The Rheonix CARD® system provides multiplexed endpoint analysis and can be rapidly customized for a wide breadth of diagnostic applications.

Rheonix CARD® Technology

Chemistry And Reagent Device

All functions are automatic

- Microfluidic System
  - All pumps, valves, microchannels, reservoirs and reaction chambers are self-contained
- True & Fully automated “sample preparation”
- Cell concentration/Cell Lysis
- DNA/RNA isolation and purification
- Molecular amplification
- Detection via Hybridization or primer extension
- Onboard readout –multiple formats
- Uniplex or Multiplex Assays
- Immunoassays
- Immunoassays AND Molecular Assays on a single Rheonix CARD® device



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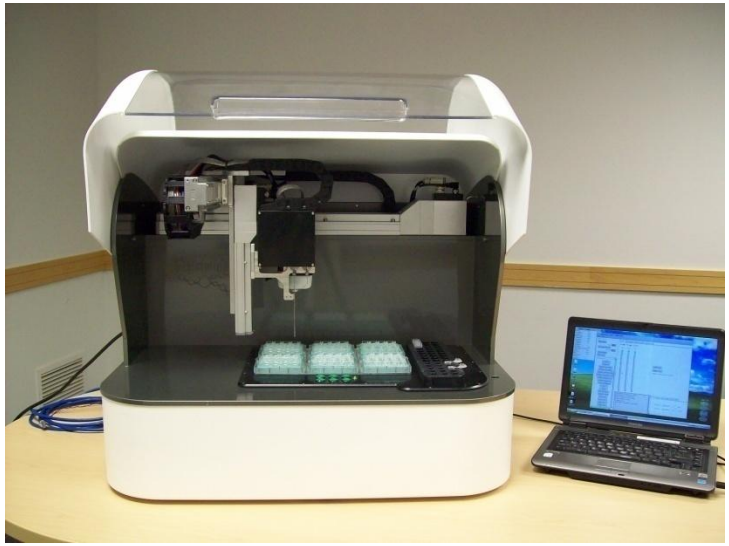
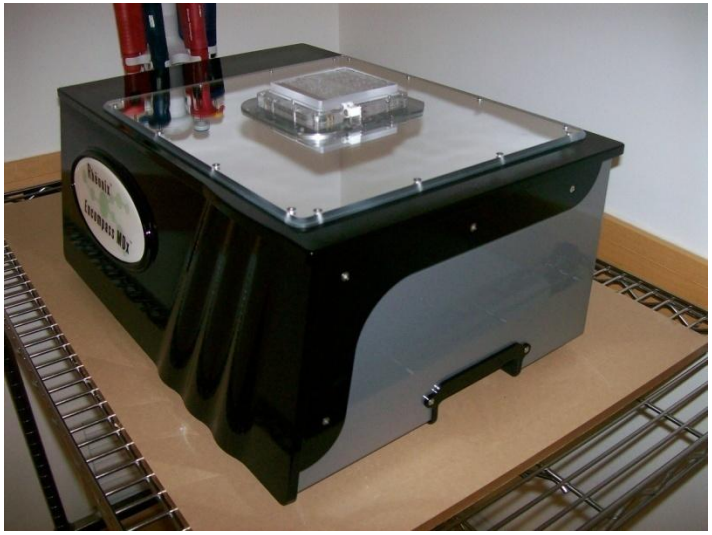
Central Lab or POU Possible

EncompassMDx™

Liquid Handler/Workstation



Portable Controller



- Regardless of instrument - no user intervention required
  - Software controlled
  - Once the “raw” sample is introduced – everything is automatic
    - Simple performance - Hit a button – get results

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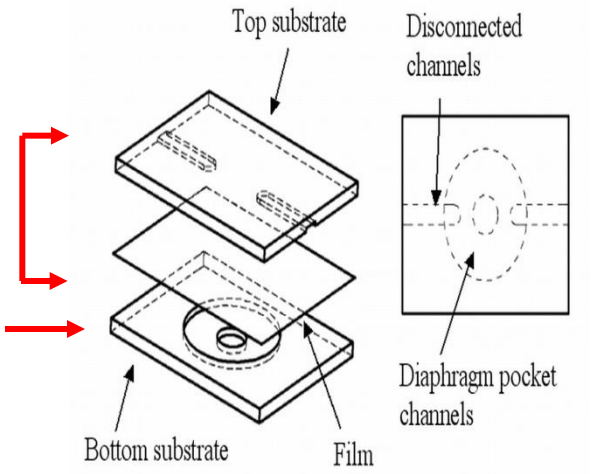
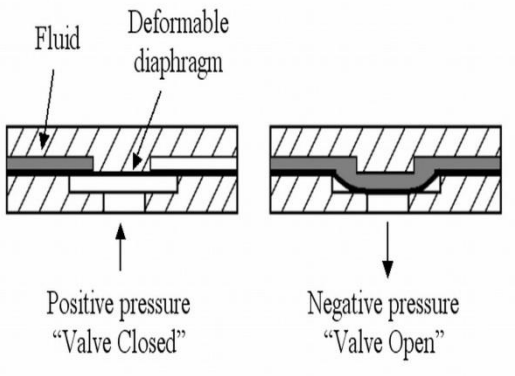
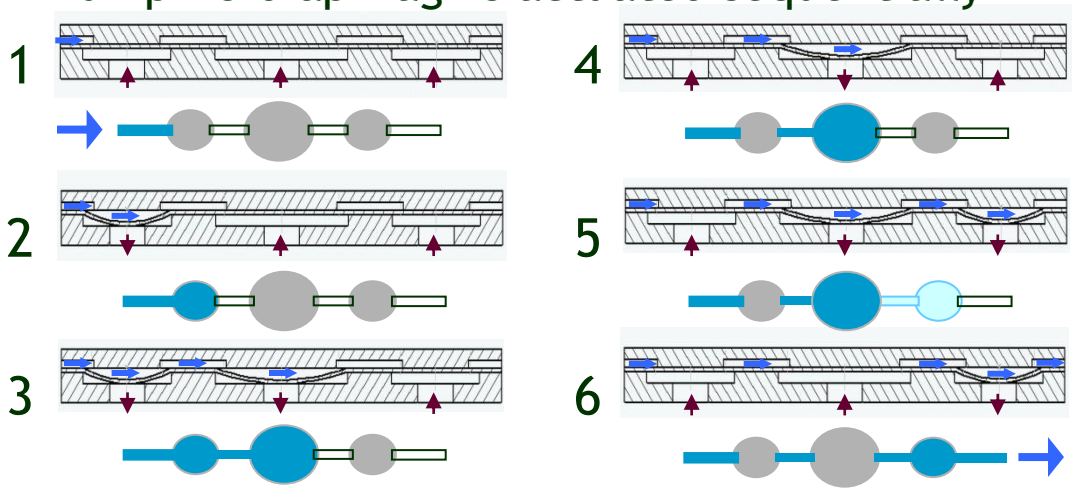
On-CARD® Valves/Pumps





Pump – 3 diaphragms actuated sequentially



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Automated Rheonix CARD® Processes

- Cells can be concentrated, if necessary (via IMS)
- Cells are lysed
  - Can be chemical or enzymatic
- DNA (or RNA) is extracted
- DNA (or RNA) is purified –orthogonal purification possible
- DNA (or RNA) is amplified
  - PCR
  - NASBA
- Amplicons are detected via reverse dot blot
  - Real time PCR also possible

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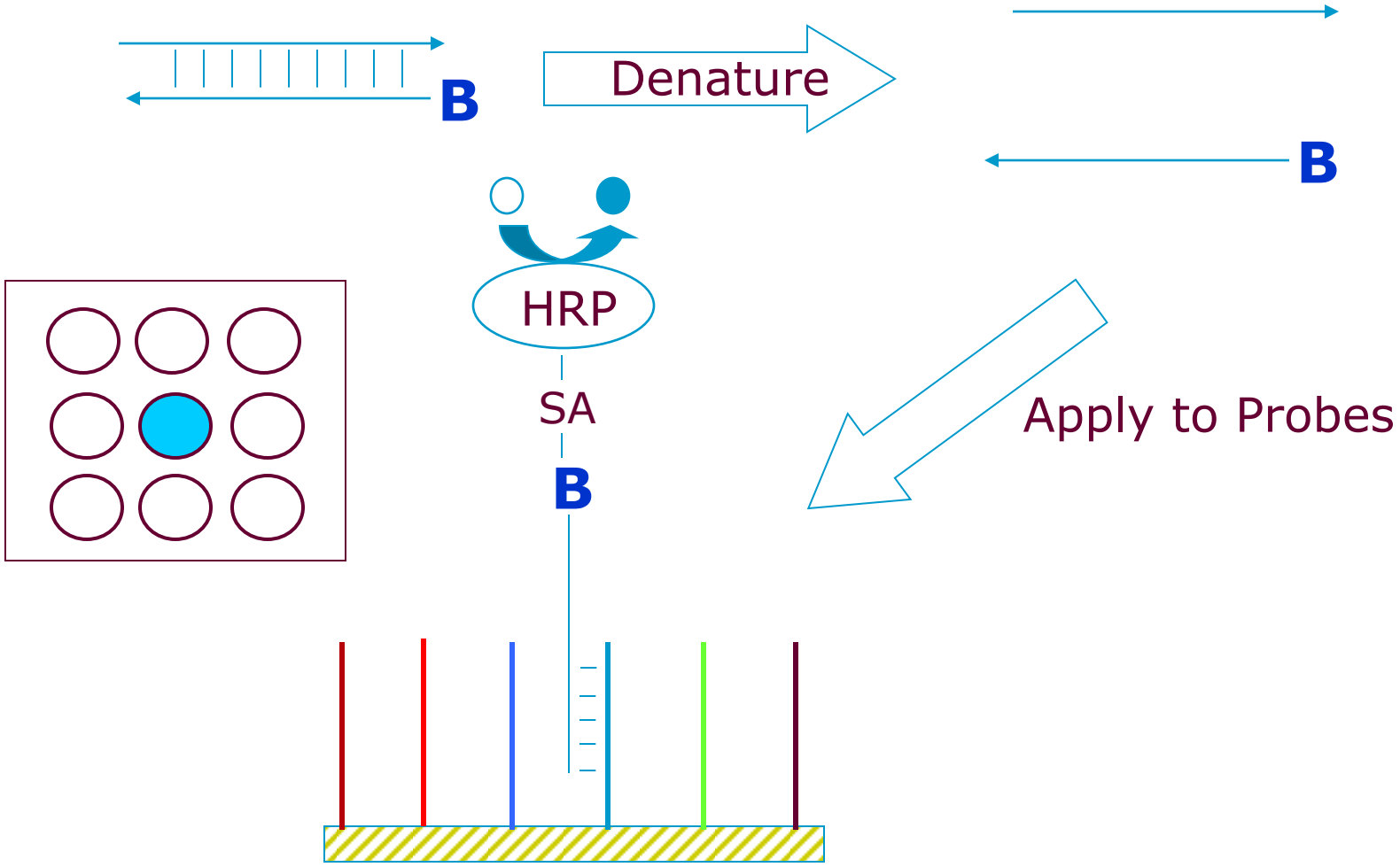
Reverse Dot Blot on CARD®



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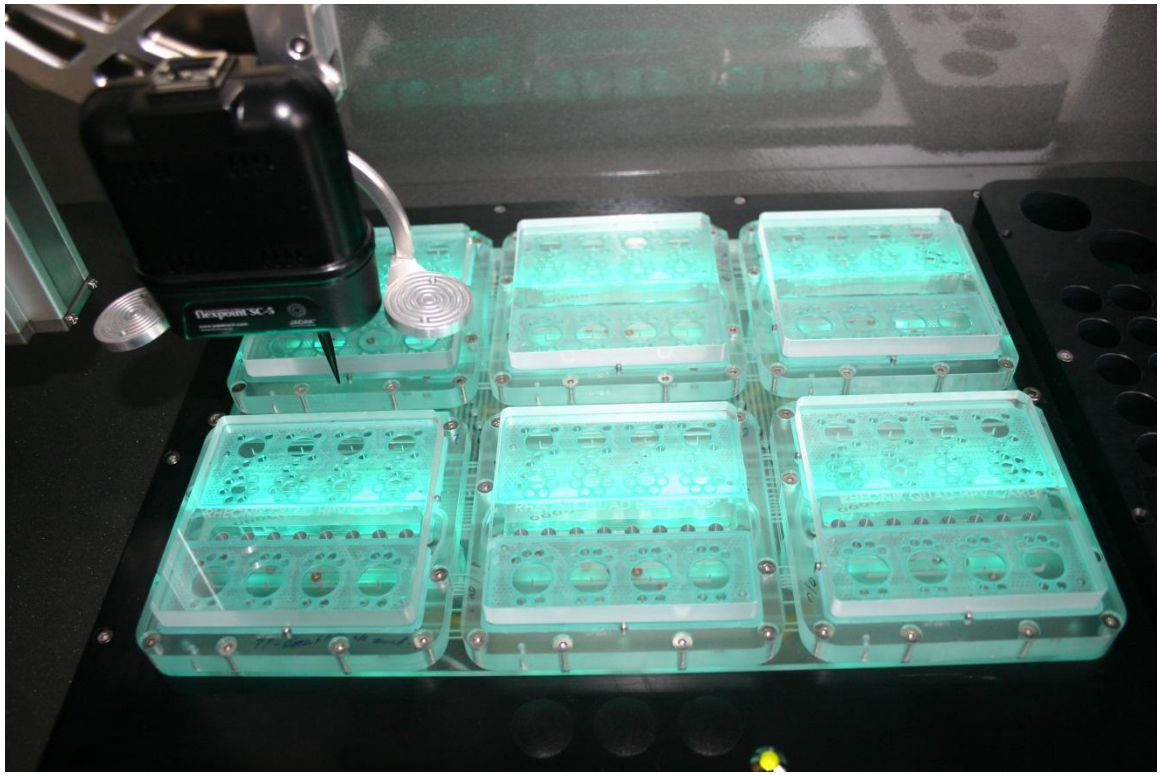
Sample Types Evaluated on Rheonix CARD® Device

- Clinical
  - Whole Blood
  - Serum
  - Plasma
  - Saliva
  - Buccal Swabs
  - Vaginal Swab
  - Whole Tissue (FFPE)
- Non-clinical
  - Drinking Water
  - Recreational Water
  - Juices, Milk, etc.



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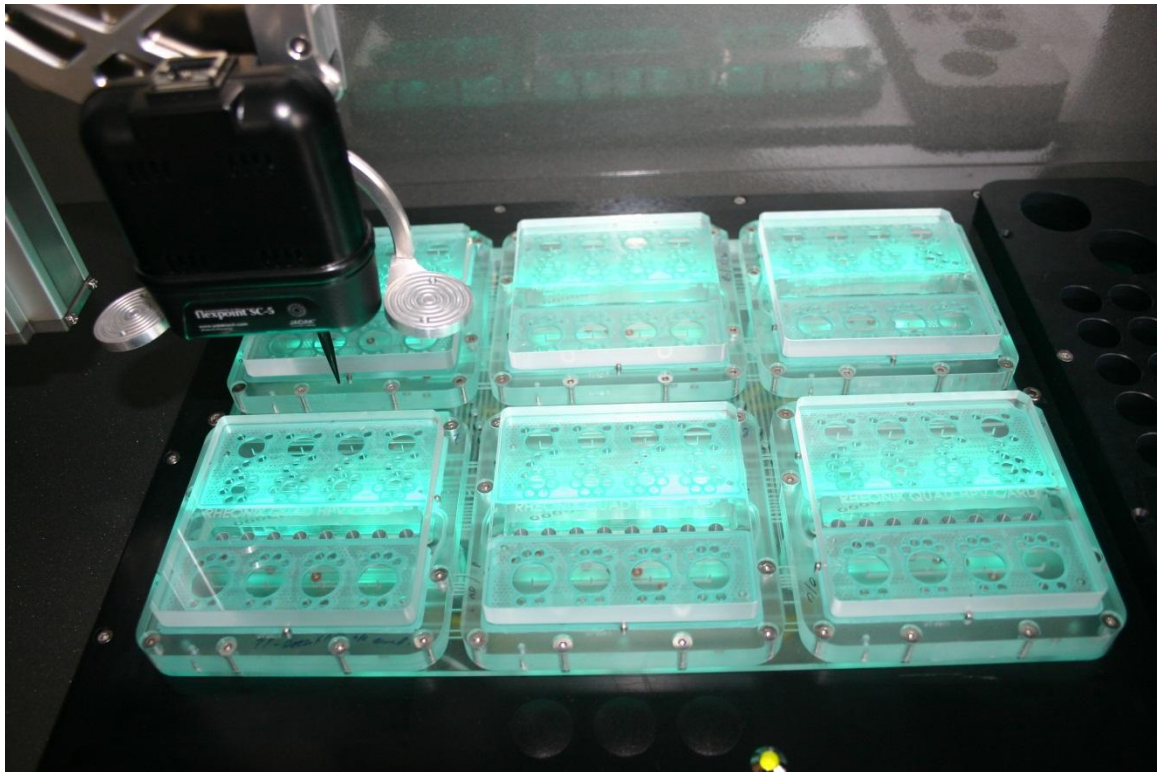

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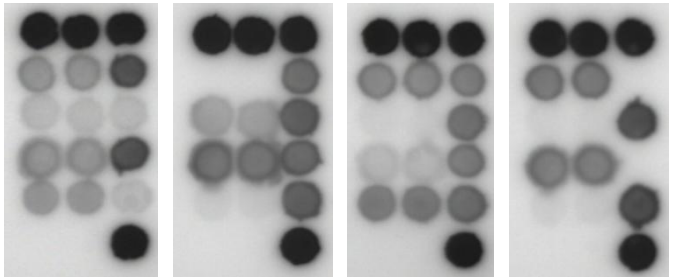
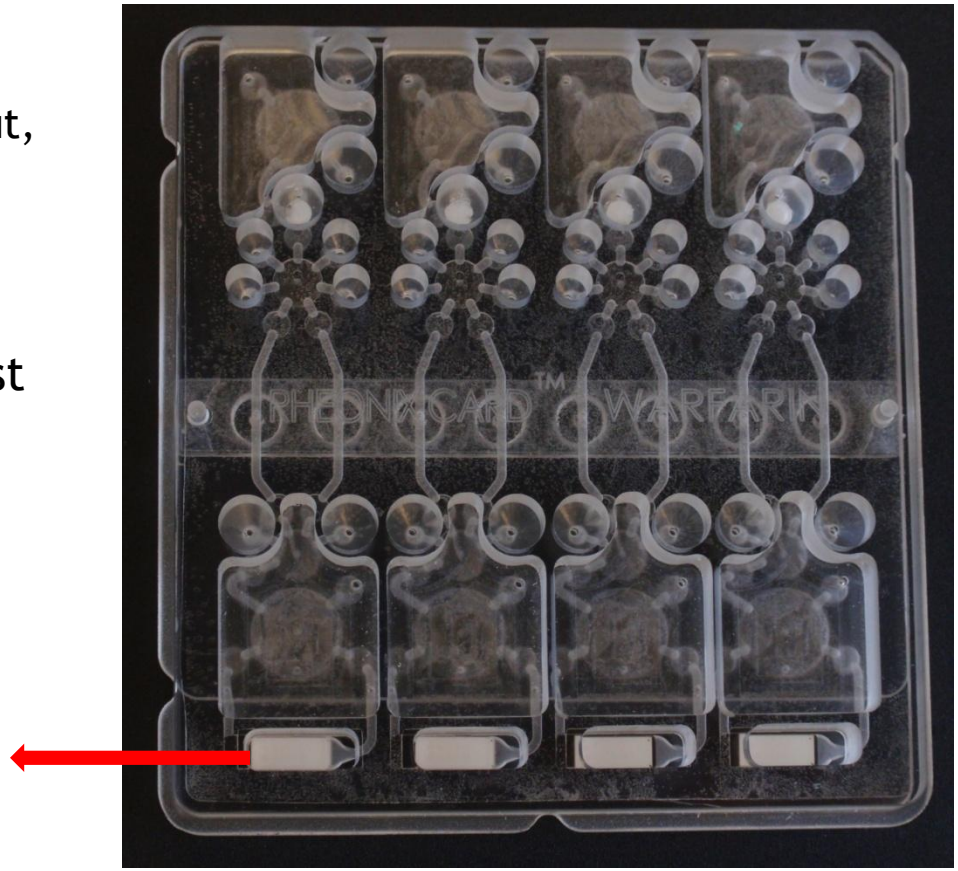
Warfarin Genotying CARD® -(SNP Assay)

- Raw sample is whole blood
- 3 SNPs evaluated
  - 3 Genotypes each (WT/WT, WT/mut, mut/mut)
- Genomic “calls” made by software
- Results have been confirmed against bi-directional sequencing.
- Will enter US clinical studies soon.





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Design Control: Assay Inputs

Parameter	Target Specification
Input sample	"Raw" beach water sample
Input Volume	100 ml
Concentrate Volume	1-2 ml, ElutraSep Hollow Fiber, 5 min
Input Volume into CARD®	1-2 ml
Time to Result	< 3.5 hours
Target Organisms	*E. coli*, *E. faecium*, *E. faecalis*, *E. gallinarum and E. avium*
Target genes	*E. coli: ECA*, 16s rRNA *Enterococci: tuf*
Detection Limit	100 CFU/100 ml E. coli 33 CFU/100 ml Enterococccci
Ability to Determine Viability Status	Yes

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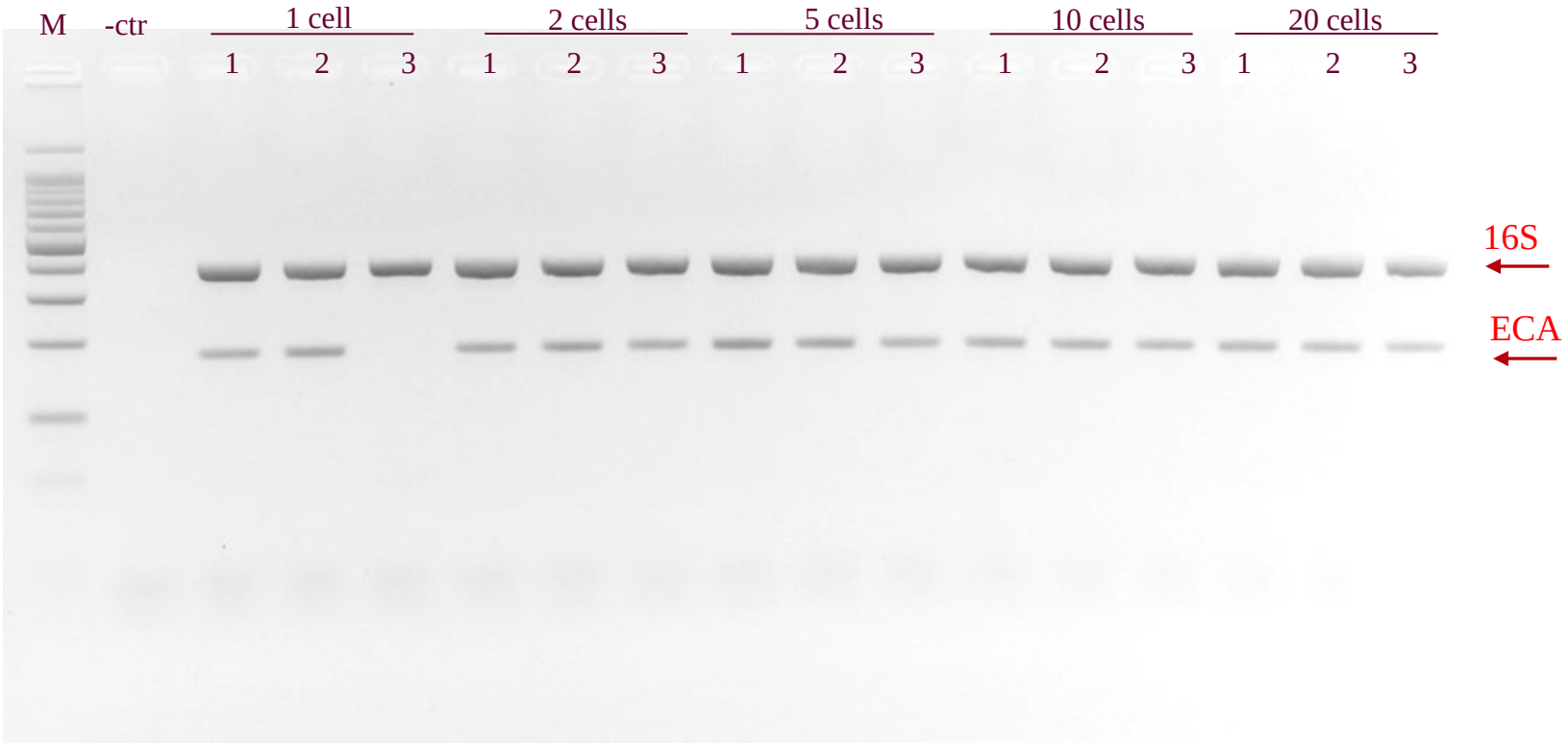
“Bench top” PCR conditions

- Bench top PCR amplification of *E. coli*
- Similar results for enterococci



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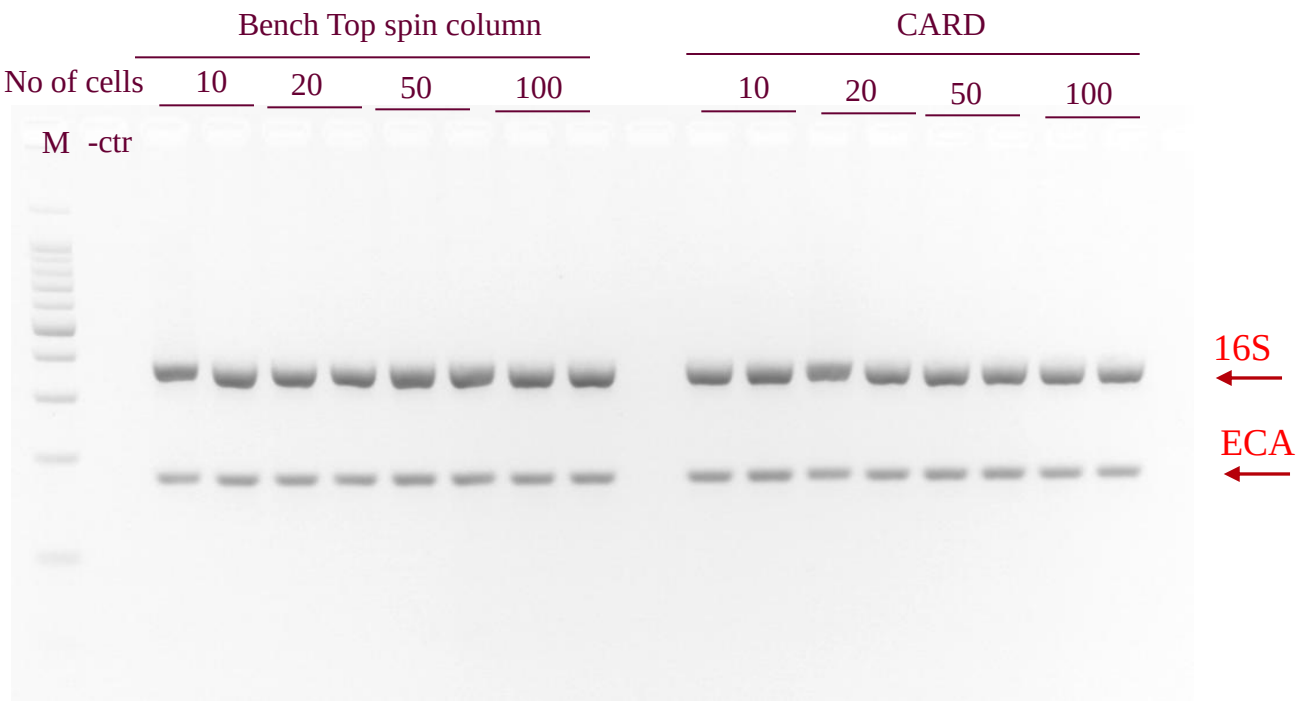
Spin column:CARD Comparison

- Isolation of DNA
- PCR Amplification



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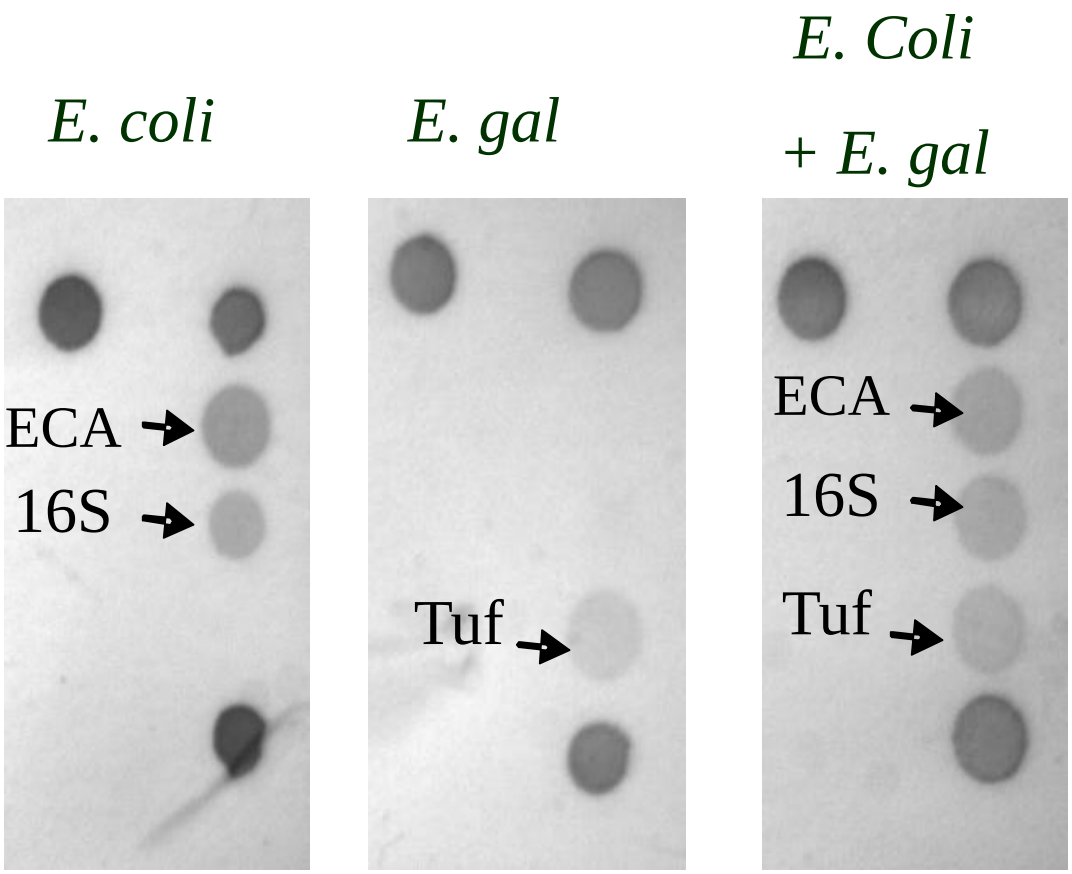
Rheonix CARD® Results



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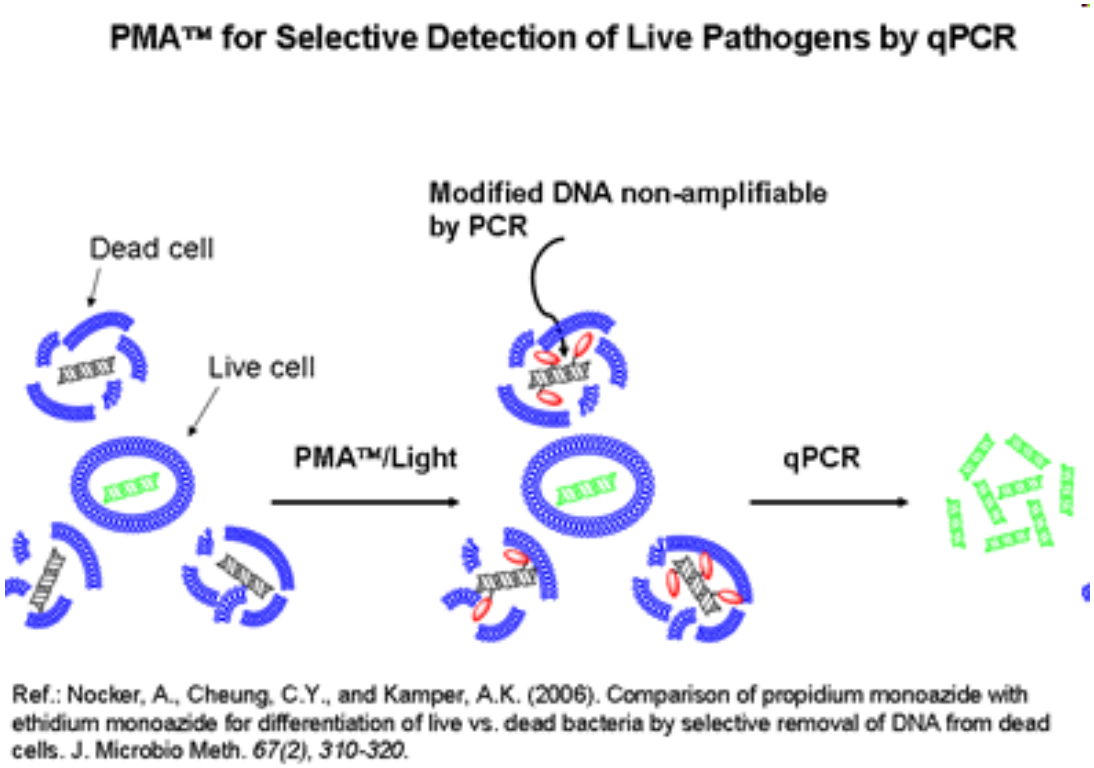
PMA: Mechanism of action



Ref.: Nocker, A., Cheung, C.Y., and Kamper, A.K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbio Meth. *67(2), 310-320.*

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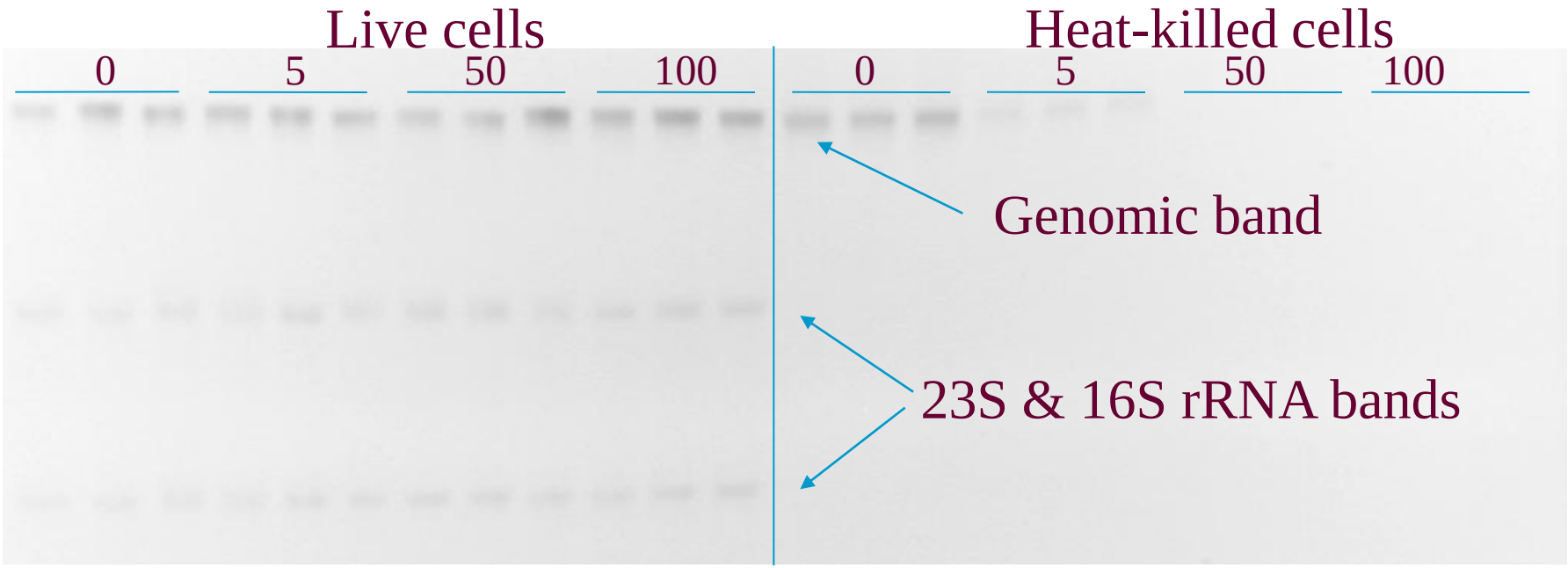
Effect of PMA on isolation of nucleic acids



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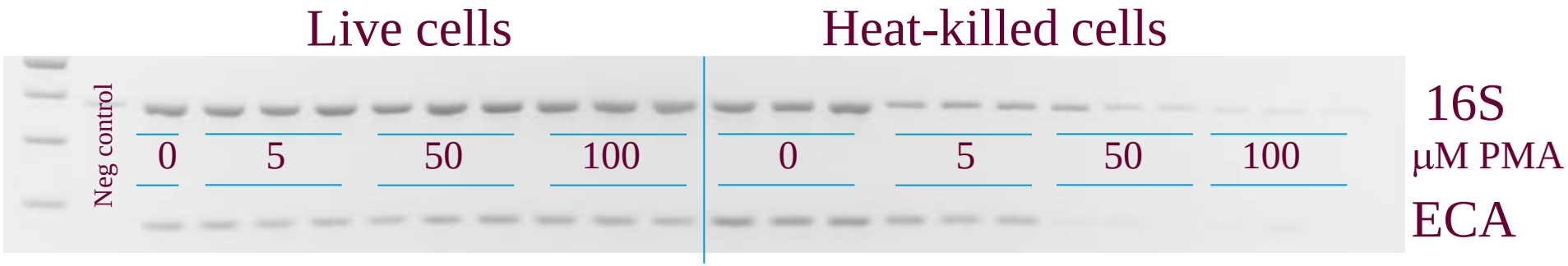

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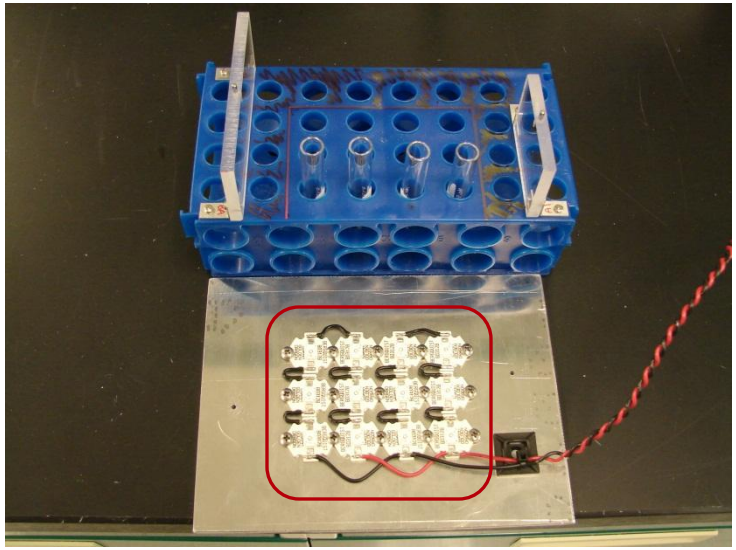
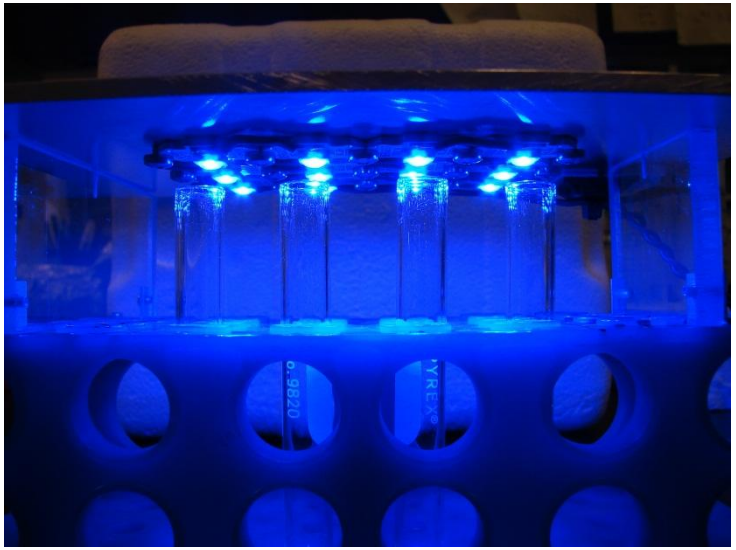
Use of PMA to establish viability

- "Bench top" feasibility studies used 600W halogen lamp
  - Heat generated required keeping samples on wet ice
  - Would not be compatible with plastic
- CARD will use blue LEDs to generate required light energy.
  - Proof of principal completed





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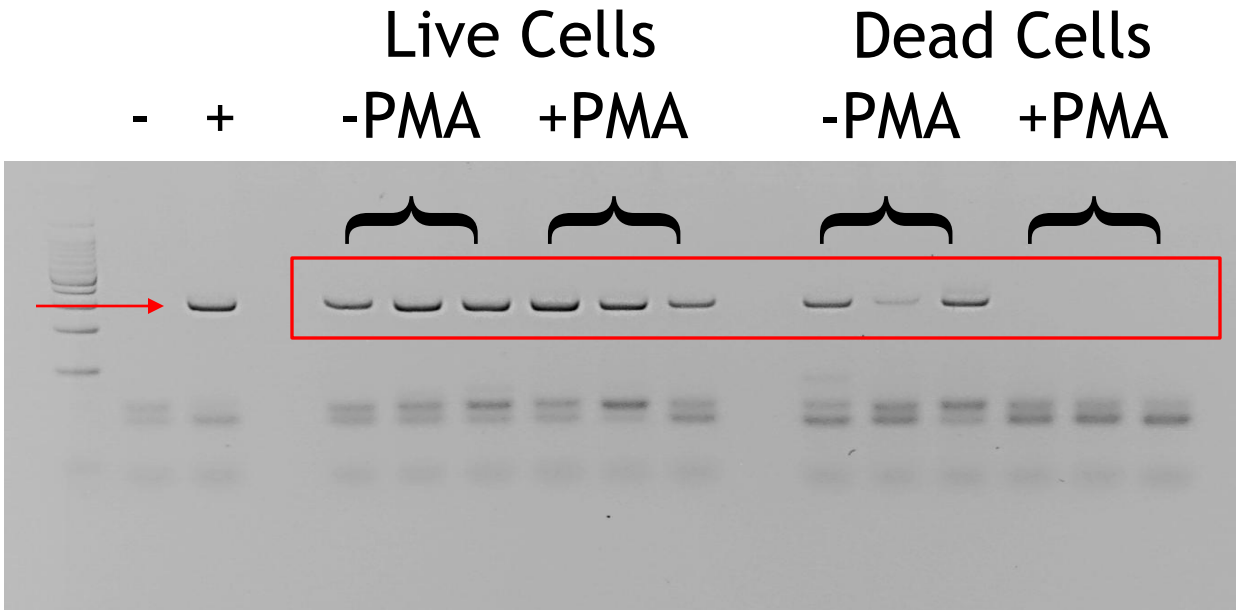
Blue Light LED Activation of PMA



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Benefits of Rheonix CARD®

- Rheonix CARD® technology can provide fully automated molecular (and/or immunologic) assays without any user intervention.
- Inexpensive instrumentation (both portable and central lab formats).
- Low cost disposable provides economical solution to POC, POU and Central lab settings.
- Assays are fully automated and controlled by software – reducing training requirements.
- Versatile platform that generally requires only a few months to migrate a “bench top” assay to the fully automated Rheonix CARD® Platform.

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